Mediterranean Journal of Basic and Applied Sciences (MJBAS)
Volume 7, Issue 2, Pages 01-09, April-June 2023

Evaluation of Changes in Coagulation Factors in Cryoprecipitate Plasma During Storage at Kisii Teaching and Referral Hospital

Collince Odiwuor Ogolla[1*], Benson Nyanchongi[1] & Silas Awuor[2]

[1]*Department of Applied Health Science, School of Health Science, Kisii University, P.O Box 408-40200, Kenya.*
[2]*Department of Medical Microbiology, Jaramogi Oginga Odinga Teaching and Referral Hospital, P.O Box 849, Kenya.*
*Corresponding Author (Collince Odiwuor Ogolla) Email: ogollacollince@gmail.com**

DOI: https://doi.org/10.46382/MJBAS.2023.7201

Article Received: 21 February 2023 *Article Accepted: 17 April 2023* *Article Published: 25 April 2023*

ABSTRACT

Background: Cryoprecipitate is used without knowing the concentration of coagulation factors it contains which might cause circulatory overload with no any improvement if several bags are transfused with low factor levels.

Objective: The objective was to assess the changes in coagulation factors in cryoprecipitate plasma during storage at -18°C for 5 weeks at Kisii Teaching and Referral Hospital.

Methods: The study involved time series analysis design involving analysis of cryoprecipitate during storage at -18°C for 5 weeks. Blood collected from the Kisii satellite blood transfusion center was received at Hematology laboratory where factor assays were performed on Erba Mannheim ECL 105 semi-automated coagulation analyzer. Thawing for subsequent coagulation factor analysis and serial testing was done using Stericox Plasma Thawing Bath at 37°C, for 45 mins. Data were entered into Excel and analyzed by SPSS version 25.

Results: The mean rank for cryoprecipitate from week one to week five was in a decreasing trend with 3.00, 1.99 and 1.01 respectively. This confirms a steady statistical significant difference in mean ranks for the time period.

Conclusion: There was a significant reduction in coagulation factors in cryoprecipitate plasma during storage at -18°C for 5 weeks at Kisii Teaching and Referral Hospital.

Keywords: Kisii Teaching and Referral Hospital; Cryoprecipitate; Coagulation factors; Time series analysis.

1. Introduction

Cryoprecipitate is a frozen blood product prepared by thawing of FFP to 1°C to 6°C and thereafter centrifuged, the precipitate is collected which is the cryoprecipitate. Cryo is rich in clotting factors which are proteins which help to slow or stop bleeding hence reducing blood loss. The factors include FI, FXIII, FVIII and vWF. Cryoprecipitate is used for the control and stoppage of bleeding in people in cases where their blood doesn't properly clot. This comprises patients experiencing grave but rare inherited disorders like Haemophilia A (deficiency of FVIII) and also von Willebrand disease (deficiency of vWF) (British Committee for Standards in Haematology Chairman) et al., 2004). Cryoprecipitate has high concentration of coagulation FVIII, FXIII, and FI (Nascimento et al., 2014).

Cryoprecipitate has similar storage condition as fresh frozen plasma (-18°C for 1 year) but cannot be re-frozen once separated as FFP. Once thawed, it is stored at room temperature for 4 hrs. Cryoprecipitate is indicated for treatment for FVIII deficiency, congenital or acquired FI deficiency, treatment for von Willebrand's Disease, FXIII deficiency, "Fibrin Glue" which is smeared to surgical sites (Rudmann, 2005).

There are several resources-limited county hospitals, referral hospitals and sub-county health centers laboratories in Kenya where factor assay are not performed systematically on patients experiencing bleeding disorders attending these facilities, cryoprecipitate and FFP is used without knowing the concentration of coagulation factors it contain which might cause circulatory overload with no any improvement if several bags are transfused with low level of coagulation factors. Blood components transfusion especially cryoprecipitate and FFP is also carried out

without prior evaluating the levels, content or quality of coagulation factors which poses risk in efficient patient management at Kisii Teaching and Referral Hospital. Thus, this study aims at evaluating blood coagulation factors and bacterial contamination in different types of plasma preparations for transfusion at KTRH.

2. Methods

2.1. Study Site

This study was conducted at Kisii Teaching and referral hospital (KTRH) laboratory department. KTRH is located within Kisii town at the southern end of the western Kenyan highlands at an altitude of 1,660m above sea level. Coordinates for the town are 0°41'S 34°46'E/0.683°S 34.767°E.

2.2. Sample Size

The study involved 108 eligible volunteer blood donors at Kisii Satellite Blood Transfusion Center, who met the donor suitability criteria following the World Health Organization guidelines.

2.3. Study Design

This study involved time series analysis design involving time series analysis of cryoprecipitate plasma during storage at -18°C for 5 weeks at an interval of one week. Four hundred- and fifty-ml blood was collected into tetra blood bags containing citrate-phosphate-adenine anticoagulant-preservative (*CPDA-1*) as an anti-coagulant preservative for subsequent processing into cryoprecipitate for storage at -18°C.

The collected blood was centrifuged at 4000 RPM for 9 minutes within 5 - 8 hours after collection in a separate sanitized room where about 180ml plasma was formed as supernatant which then was separated and collected. The 180ml plasma obtained through centrifugation was aliquoted in three parts each containing 60 ml. The first aliquot was used to assess the changes in coagulation factors in cryoprecipitate plasma at room temp at baseline during week one of collection (baseline), the second aliquot was used to assess the changes in coagulation factors in cryoprecipitate plasma storage at -18°C temp after three weeks of storage, the third aliquot was used to assess the changes in coagulation factors in cryoprecipitate plasma storage at -18°C temp after five weeks of storage.

Coagulation factor analysis was performed using Erba Mannheim ECL 105 coagulation analyzer, India at KTRH Hematology laboratory. Thawing for subsequent coagulation factor analysis and serial testing of stored fresh frozen plasma and cryoprecipitate was done using Stericox Plasma Thawing Bath, an equipment designed for rapid and uniform thawing of fresh frozen plasma (FFP) bags at 37°C, for 45 mins before the samples are analyzed by Erba Mannheim ECL 105 coagulation analyzer, India and results recorded to assess the coagulation factors changes and levels in cryoprecipitate. Standard storage conditions for the aliquots were observed and maintained to ensure their coagulation factor levels homogeneity.

3. Data management and statistical analysis

The data was recorded as numbers (value measured). Statistical analysis was descriptive statistics. The raw data collected was entered in Microsoft office Excel spreadsheet before being transferred to SPSS software version 25.0. The findings were presented in tables and graphs.

4. Ethical Considerations

Institutional ethical clearance was obtained from Baraton ethical review committee (UEAB/ISERC/02/05/2022) and research permit obtained from National Commission for Science and Technology (NACOSTI) - NACOSTI/P/22/17542.

5. Results

For the realization of the results, Friedman test analysis was employed to establish the changes in coagulation factors in cryoprecipitate plasma. The results were as shown below.

Table 1. Descriptive Statistics for Coagulation Factors in Cryoprecipitate Plasma

Variables	N	Mean	Std. Deviation	Minimum	Maximum
CRYOPW1	108	119.10	19.96	61.75	157.25
CRYOPW3	108	109.81	19.22	56.00	148.25
CRYOPW5	108	100.28	18.83	52.50	138.50

Coagulation factors in cryoprecipitate plasma in Week 1 (CRYOW1), Coagulation factors in cryoprecipitate plasma in Week 3 (CRYOW3) and Coagulation factors in cryoprecipitate plasma in Week 5 (CRYOW5).

The mean of coagulation factors in cryoprecipitate plasma for week one was 119.10 with a standard deviation of 19.96. The third week presented a mean of 109.81 with a standard deviation of 19.22. Findings of the fifth week of the study showed that the mean was 100.28 and the standard deviation of 18.83. This means that most of the coagulation factors in cryoprecipitate plasma across the five-week study did not vary widely, but were distributed around the central value which is the mean of the 108 blood donors. From the mean values and standard deviation values it was clear that there was a significant difference in coagulation factors for the period, thus, the normality aspect was observed.

Table 2. Chi-square Test for Influence of Gender on Coagulation Factors in Cryoprecipitate Plasma

			Gender		Chi-square Test		
			Male	Female	Degree of Freedom	Chi-square value	P-value
W1F1	Normal	Count	49	49	1	1.454	0.228
		% of Total	45.4%	45.4%			
	Abnormal	Count	7	3			
		% of Total	6.5%	2.8%			
W1FVIII	Normal	Count	53	51	1	0.892	0.345
		% of Total	49.1%	47.2%			

	Abnormal	Count	3	1			
		% of Total	2.8%	0.9%			
W1FXIII	Normal	Count	54	50	1	0.006	0.940
		% of Total	50.0%	46.3%			
	Abnormal	Count	2	2			
		% of Total	1.9%	1.9%			
W1 Von Willebrand	Normal	Count	53	48	1	0.243	0.622
		% of Total	49.1%	44.4%			
	Abnormal	Count	3	4			
		% of Total	2.8%	3.7%			
W3F1	Normal	Count	54	50	1	0.006	0.940
		% of Total	50.0%	46.3%			
	Abnormal	Count	2	2			
		% of Total	1.9%	1.9%			
W3FVIII	Normal	Count	55	52	1	0.937	0.333
		% of Total	50.9%	48.1%			
	Abnormal	Count	1	0			
		% of Total	0.9%	0.0%			
W3FXIII	Normal	Count	55	52	1	0.937	0.333
		% of Total	50.9%	48.1%			
	Abnormal	Count	1	0			
		% of Total	0.9%	0.0%			
W3 Von Willebrand	Normal	Count	54	51	1	0.271	0.602
		% of Total	50.0%	47.2%			
	Abnormal	Count	2	1			
		% of Total	1.9%	0.9%			
W5F1	Normal	Count	53	52	1	2.865	0.091
		% of Total	49.1%	48.1%			
	Abnormal	Count	3	0			
		% of Total	2.8%	0.0%			

W5FVIII	Normal	Count	54	51	1	0.271	0.602
		% of Total	50.0%	47.2%			
	Abnormal	Count	2	1			
		% of Total	1.9%	0.9%			
W5FXIII	Normal	Count	54	52	1	1.892	0.169
		% of Total	50.0%	48.1%			
	Abnormal	Count	2	0			
		% of Total	1.9%	0.0%			
W5 Von Willebrand	Normal	Count	55	51	1	0.003	0.958
		% of Total	50.9%	47.2%			
	Abnormal	Count	1	1			
		% of Total	0.9%	0.9%			

From the results above, week one Factor one showed that, 49 (45.4%) of male had normal range test while only 7 (6.5%) were abnormal range and 49 (45.4%) representing those female donors who had normal range test with only 3 (2.8%) for the abnormal range test. Week five of the study showed that, 55 (50.9%) were male who had normal range test for the cryoprecipitate plasma with only 1 (0.9%) representing abnormal range and 51 (47.2%) for the female had normal range test with only 1 (0.9%) indicating abnormal range.

The coagulation factors in cryoprecipitate plasma in the donated blood were not affected by the gender of the blood donor. This was shown by the results of the p-value as from week one to week five of the study (p-value>0.05). Thus, the gender of the donor has no effect on the coagulation factors in cryoprecipitate plasma.

Friedman's ANOVA Test Analysis for Coagulation Factors in Cryoprecipitate Plasma

By the use of the mean rank test of the Friedman test analysis, the study was able to establish the differences in time period for coagulation factors in cryoprecipitate plasma of the donated blood. The results were as shown below.

Table 3. Friedman's Test for Mean Rank

Variables	Mean Rank
CRYOPW1	3.00
CRYOPW3	1.99
CRYOPW5	1.01

The mean rank for CRYOPW1 to CRYOPW5 was in a decreasing trend with 3.00, 1.99 and 1.01 respectively. This confirms a steady significant difference in mean ranks for the time period though in a decreasing trend thus, the coagulations factors reduce as the time goes by.

Table 4. Friedman's ANOVA Test Analysis

Test Statistics[a]	
N	**108**
Chi-Square	214.019
Df	2
Asymp. Sig.	.000

a. Friedman's Test

By establishing the statistical significance difference between the three-selected periods of coagulation factors in cryoprecipitate stored at minus 18°C for a period of 5 weeks, the Friedman's test statistics was used.

The result from Table 4.13 above shows a significant difference between the three-selected times for the stored blood at KTRH. As shown by the chi-square value of 214.019 with p-value (0.000) less than 0.05 standard alpha values. This indicates that, as the donated blood continues to be stored, there is a significant reduction in coagulation factors in cryoprecipitate plasma at -18°C for storage before transfusion.

The same can be seen in Table 4.12 above about the mean rank for coagulation factors in cryoprecipitate plasma.

Table 5. Wilcoxon Signed Ranks Test

Test Statistics[a]			
	CRYOPW3 - CRYOPW1	**CRYOPW5 - CRYOPW1**	**CRYOPW5 - CRYOPW3**
Z	-9.022[b]	-9.022[b]	-9.019[b]
Asymp. Sig. (2-tailed)	.000	.000	.000

a. Wilcoxon Signed Ranks Test

b. Based on positive ranks.

Wilcoxon signed rank test was used to indicate specific difference between the five-week timeframe for coagulation factors in cryoprecipitate plasma for the three selected weeks.

The results shown in Table 4.14 above indicates a significant difference when the two variables were compared, that's; CRYOPW1 – CRYOPW3, CRYOPW1 – CRYOPW5 and lastly CRYOPW3 – CRYOPW5 all with p-value less than 0.05, thus they were significantly differed from one week to another.

Mediterranean Journal of Basic and Applied Sciences (MJBAS)
Volume 7, Issue 2, Pages 01-09, April-June 2023

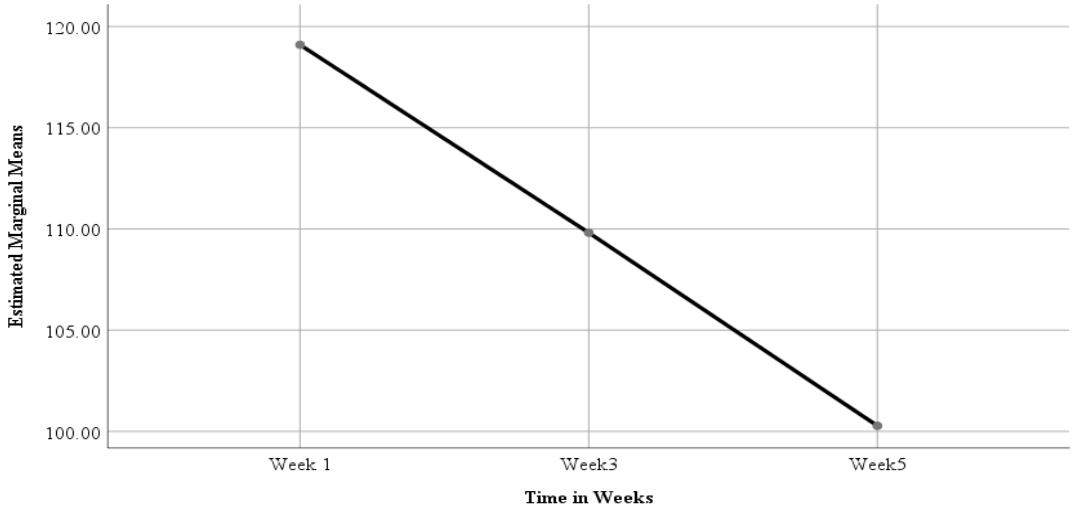

Figure 1. Estimated Marginal Means for Fresh Frozen Plasma

The diagrammatic representation results of the difference in the coagulation factors in cryoprecipitate plasma for the donated blood were as shown in Figure 4.9 above, this shows that there existed a steady and constant decrease in the coagulation factors as from week one with an approximated estimated marginal mean value of 119 to 109 for the third week and an estimated marginal mean value of 101 for the fifth week. Hence, the results relate to those shown above in Table 4.12 of the mean rank for three weeks selected for the study.

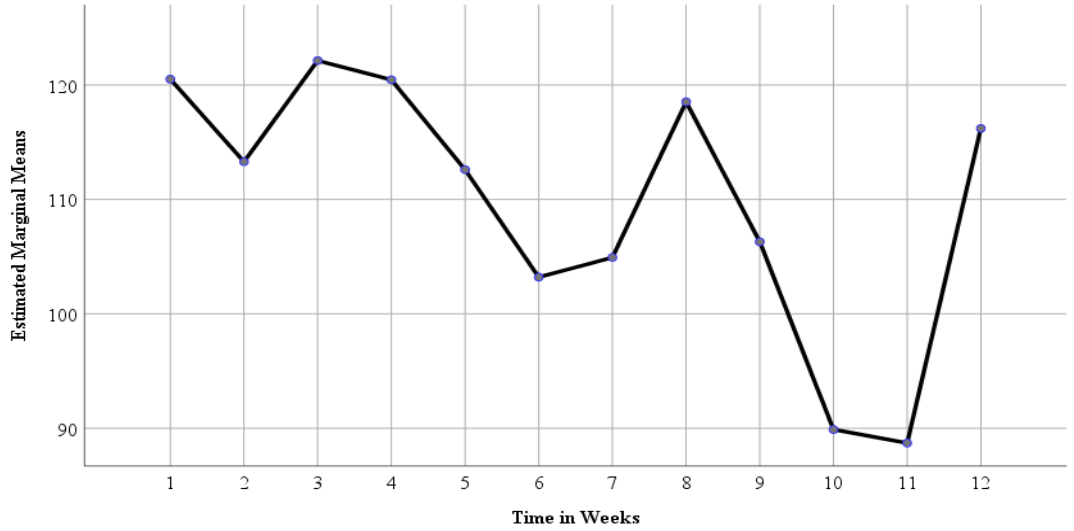

Figure 2. Estimated Marginal Means for Specific Coagulation Factors in Fresh Frozen Plasma

The specific coagulation factors in cryoprecipitate plasma of the donated blood ready for transfusion, each week was represented by four factors that's; 1 (Week1 FI), 2 (Week1 FVIII), 3 (Week1 FXIII), 4 (Week1 Von. Willebrand), 5 (Week3 FI), 6 (Week3 FVIII), 7 (Week3 FXIII), 8 (Week3 Von. Willebrand), 9 (Week5 FI), 10 (Week5 FVIII), 11 (Week5 FXIII), 12 (Week5 Von. Willebrand). The results in the line graph above of estimated marginal means and time in weeks for the coagulation factors in cryoprecipitate plasma shows that, at the end of the last week there was a decrease of coagulation factors FI with approximated estimated marginal mean value of

about 121 for the first week, and to the factor FXIII of week five with an estimated marginal mean value of 89 with an increase in coagulation factors in Von. Willebrand to 116 estimated marginal mean value which was still less than that of FI of week one. The results means that, with standardized storage conditions at the blood bank at KTRH, the coagulation factors in cryoprecipitate plasma were reducing though with a significant decrease.

6. Discussion

The mean of coagulation factors in cryoprecipitate plasma for the first week of the study being at 119.10 with a standard deviation of 19.96 with the other weeks recording a change which was significant. This means that most of the coagulation factors in cryoprecipitate plasma across the five-week study did not vary widely, but were distributed around the mean value hence a significant difference in coagulation factors for the period of the study. For the mean rank for cryoprecipitate plasma for week one to cryoprecipitate plasma for week 5 was in a decreasing but significant trend with 3.00, 1.99 and 1.01 respectively. To test the statistical significance difference between the three selected periods of coagulation factors in cryoprecipitate plasma, the freedman's test statistics was used. There existed a statistically significant difference between the three selected time periods for the stored blood with chi-square p-value (0.000). This finding is anchored by (Green, Backholer, et al., 2016) that storing of blood after donation at given storage conditions, the coagulation factors in cryoprecipitate plasma decreases though significantly with a decreasing rate of less ten percent averagely which in support of the study results as shown. This means that, as the donated blood continues to be stored, there is a significant reduction in coagulation factors in cryoprecipitate plasma at -18°C for storage before transfusion. The same results were found by (Cid et al., 2013) that cryoprecipitate plasma prepared contained significantly declined levels of coagulation factors. In-addition, the findings of (Kovacic Krizanic et al., 2022) found that reduction in coagulation factor levels in cryoprecipitate plasma was statistically significant.

7. Conclusion

There was a significant reduction in coagulation factors in cryoprecipitate plasma during storage at -18°C for 5 weeks at Kisii Teaching and Referral Hospital, Kisii County.

Declarations

Source of Funding

This research work did not receive any grant from funding agencies in the public or not-for-profit sectors.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Consent for Publication

The authors declare that they consented to the publication of this research work.

Availability of Data and Materials

All relevant data are shared on this manuscript

Ethical Approval

Institutional ethical clearance was obtained from Baraton ethical review committee (UEAB/ISERC/02/05/2022) and research permit obtained from National Commission for Science and Technology (NACOSTI) - NACOSTI/P/22/17542.

Acknowledgements

(a) Special appreciation to my mentors and supervisors of this research project, Dr. Benson Nyanchongi (PhD) and Dr. Rodgers Norman Demba (PhD). (b) Special thanks to lecturers in the department of biomedical science for the skills and knowledge gained. (c) Special thanks to KTRH, Kisii University and NACOSTI for the opportunity they gave me to carry out this study. (d) My special thanks also go to my family, parents (Hellen Ogolla and Joseph Ogolla) and siblings without whom this incredible experience would not have been possible.

References

[1] J.D., O'Shaughnessy, D.F., Atterbury, C., Bolton Maggs, P., Murphy, M., Thomas, D., Yates, S., & Williamson, L.M. (2004). Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. British Journal of Haematology, 126(1): 11–28.

[2] Nascimento, B., Goodnough, L.T., & Levy, J.H. (2014). Cryoprecipitate therapy. British Journal of Anaesthesia, 113(6): 922–934.

[3] Rudmann, S.V. (2005). Textbook of blood banking and transfusion medicine. Elsevier Health Sciences.

[4] Green, L., Backholer, L., Wiltshire, M., Platton, S., Stanworth, S.J., & Cardigan, R. (2016). The hemostatic properties of thawed pooled cryoprecipitate up to 72 hours. Transfusion, 56(6): 1356–1361.

[5] Cid, J., Caballo, C., Pino, M., Galan, A.M., Martínez, N., Escolar, G., & Diaz-Ricart, M. (2013). Quantitative and qualitative analysis of coagulation factors in cryoprecipitate prepared from fresh-frozen plasma inactivated with amotosalen and ultraviolet A light. Transfusion, 53(3): 600–605.

[6] Kovacic Krizanic, K., Prüller, F., Rosskopf, K., Payrat, J.-M., Andresen, S., & Schlenke, P. (2022). Preparation and Storage of Cryoprecipitate Derived from Amotosalen and UVA-Treated Apheresis Plasma and Assessment of In Vitro Quality Parameters. Pathogens, 11(7): 805.